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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
KYRKANIDES, Stephanos	)	Art Unit: 1632
	)	
Application No. 10/781,142	)	Examiner: Hama, Joanne
	)	
Filing Date: February 18, 2004	)	Confirmation No. 3987
	)	
For: VECTORS HAVING BOTH ISOFORMS OF B-HEXOSAMINIDASE AND USES OF THE SAME	)	

DECLARATION OF STEPHANOS KYRKANIDES UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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I, Stephanos Kyrkanides, a citizen of U.S., residing at 101 Country Club Drive, Rochester, NY 14618, declare that:

1. I am a Associate Professor in the departments of Dentistry, Neurobiology & Anatomy, and Center for Oral Biology at the University of Rochester Medical Center, Rochester, NY. I am the Chair and Program Director of Orthodontics and Dentofacial Orthopedics and the Coordinator of the Craniofacial Research Program at University of Rochester Medical Center. I hold a Ph.D. from University of Rochester, Rochester, N.Y.; a D.D.S. from University of Athens, Greece; and a M.S. from University of Rochester. I have over eight years experience in the field of craniofacial development. This includes specific experience in the study of the development of gene therapy for the treatment of congenital craniofacial malformations due to inherited metabolic diseases. A partial curriculum vitae is attached to this declaration as an exhibit.

2. I am an inventor in the above referenced application relating to bi-cistronic vector constructs comprising nucleic acids encoding both HEX-α and HEX-β and demonstrating the use of these constructs to produce functional Hexosaminidase B protein, which is a heterodimer of HEX-α and HEX-β.

3. I have reviewed the findings of Tian and Skolnick, wherein the conservation of enzyme function was evaluated as a function of pairwise sequence identity to demonstrate the predictability of enzyme function for sequence variants. Specifically, Tian and Skolnick evaluated the predictability of the enzyme commission (EC) number for proteins based on sequence identity. The findings of Tian and Skolnick indicate that 90% of enzyme mutants in nature will maintain enzyme function with sequence identities as low as 60% and in fact enzyme function does not generally *start* to diverge until the sequence identity is below 70% (see Tian and Skolnick, J. Mol. Biol. 2003. Oct 31;333(4):863-82, abstract, page 863). This article evidences the recognition in the field of enzymology that enzymes are envisioned as having a range of variants that are expected to be functional. More specifically, based on these findings, one would generally have a high expectation that an enzyme, such as HEX-α or HEX-β, having 70% sequence identity to the wild-type enzyme would possess at least some enzymatic activity.

4. Furthermore, much was known about the structure-function relationship for HEX-α and HEX-β at the time the instant application was filed. For example, it was known at the time this application was filed that amino acids 1-191 and 403-529 of HEX-α and amino acid 225-556 of HEX-β are required for G_{M2} substrate specificity (Pennybacker, M., et al., 1996. J. Biol. Chem. 271(29):17377-82). Additionally, many of the mutations in HEX-α and HEX-β that are known to cause neurological disorders were published prior to the filing of the instant application. For

example, mutations in HEX-α that were known to result in Tay-Sachs disease include a 4bp insertion in exon 11; 2bp deletion in exon 5; intron mutations; early termination codons; and Glu482Lys, 1510delC, Arg178His, Arg178Cys, Gly269Ser, Arg504His, Arg499His, Arg170Gln, Trp420Cys, Gly250Asp, Phe304Del, Arg504Cys, Ser210Phe, Arg137Ter, Arg393Ter, TRP26Ter, Arg178Leu, Met1Val, Arg499Cys, Trp329Ter, Trp485Arg, Tyr180Ter, Arg247Trp, Val192Leu, Val200Met, Asp258His, Arg170Trp, Lys197Thr, Phe211Ser, Leu127Arg, His204Arg, Met301Arg, Cly454Ser, Leu39Arg, Trp392Ter, Gly805Ala, Tyr180His, Trp474Cys, Leu451Val, and Val324Val mutations (see OMIM[1] database # 606869). Likewise, mutations in HEX-β that were known to result in Sandhoff's disease include a 16-kb deletion of the promoter, exons 1 through 5, and part of intron 5; intron mutations; and Tyr456Ser, Pro417Leu, Lys121Arg, Arg505Gln, Pro405Leu, Ala543Thr, Ser62Leu, Pro504Ser, and 76delA mutations (see OMIM database # 606873). As these disease-causing mutations were published between 1975 and 1997, someone working in the field of my patent application would at the time the instant application was filed have known to conserve a least the above amino acids, or known how to determine these amino acids, in selecting variants of HEX-α and HEX-β.

5. Moreover, a person working in the field of my patent application would have know how to test such variants of HEX-α and HEX-β for functionality (e.g., G_{M2} catabolysis). For example, the instant specification demonstrates that enzyme binding to substrate can be measured using synthetic substrates such as MU-GlcNAc and MU-FlcNac-6-SO4 (see specification page 163, line 26). Likewise, assays were known at the time the application was filed for assaying the

[1] OMIM™ - Online Mendelian Inheritance in Man™ (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM) is a catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins and elsewhere, and developed for the World Wide Web by NCBI, the National Center for Biotechnology Information.

degradation of G_{M2} gangliosides. For example, 3H-G_{M2} ganglioside and GM2 activator protein can be used to detect G_{M2} catabolysis (see Pennybacker, M., et al.).

6. Thus, I believe that someone working in the field of my patent application at the time the application was filed would have been able to envision variants of HEX-α and HEX-β of at least 95%, 80%, 70% sequence identity to the native sequences with a high probability of success that they would form functional Hexosaminidase proteins.

7. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

Date: 1/2/08

Stephanos Kyrkanides, D.D.S., Ph.D.